User Instructions

X-Lyz™ Protein Amine Assay

Catalog # IC2860101-Trial

Features:

- ***15min quick assay***
- ***Compatibility with reducing agents***
- ***Wide linear range***
- ***Suitable for microplates and cuvettes***

For safely handling the reagents, refer to the MSDS sheets online:
www.x-lyz.com

Contents

1 Product Description

Among post translational modifications (PTM), the protein amine (lysine and N-terminus) is one of the most frequently observed PTM sites. The ubiquitination process alters the fate of proteins by attaching ubiquitin molecules to unblocked amines. Its abnormal behavior may be an early indication of many disease development. During the food/feedstuff processing, the reactive amine of proteins is modified by Maillard reaction that changes the bioavailability of Lysine, and compromises their nutritional values. Therefore, it is necessary to have a reliable and simple analytical tool to measure the amount of unblocked and reactive amines in proteins.

The X-Lyz™ Protein Amine Assay Kit is an amine-reactive reagent for the quantitative determination of primary amine contents in proteins. The water-compatible reagent reacts with the primary amines in proteins or peptides to form a sensitive chromophore; its UV absorbance at 350nm is linearly proportional to the amine concentrations over a wide analytical range (25-6000nmol/mL of amines, or 25-10,000µg/mL of proteins). The reactive amine amount is quantified against the standard curve of Norvaline. The method is suitable for assays in microplates and test tubes (cuvettes).

The X-Lyz™ Protein Amine Assay Kit, Catalog # IC286101-Trial, is sufficient for up to 400 assays in microplates and 60 assays in test tubes. The contents are specified in the table below.

Table 1: Contents in X-Lyz™ Assay Kit (Cat No. IC286101-Trial)

Items	Cat No.	Size	Contents	Store	Ship
Reagent A	IC286091	25mL	Containing Reagent A in aqueous solution, pH ~10.5	Room Temp	Room Temp
Reagent B	IC286092	5mL	Containing Reagent B in organic mixture	4ºC	
Norvaline Standard	IC286097	1mL	Containing norvaline 50µmol/mL in 10mM HCl	4ºC	
Instructions	Pub286102	N/A	Instructions for X-Lyz™ Protein Amine Assay	N/A	N/A

All reagents expire one year from the date of purchase.

The assay simply involves mixing the Working Reagent (WR, prepared by combining reagent A and B at a ratio of 5/1) with samples, incubating for 15min before UV measurement at 350nm. The WR/Sample ratio of 10/1 and 1/1 may be used for different concentration ranges. A solid protein sample can also be analyzed upon dissolution in the WR.

2 Preparation of Standards and Blank

Blank and diluted Norvaline standards will be used to generate a standard curve. Use Table 2 & 3 as a guide to prepare the Blank and diluted Norvaline standards. Starting with the provided Norvaline standard (50µmol/mL, Cat No. IC286097), dilute into the desired concentrations, preferably using the same diluent as the sample(s). The Blank contains only the diluent without Norvaline. The volumes shown in the tables are sufficient for three replications of each dilution.

Table 2: Preparation of Diluted Norvaline Standards for WR/Sample Ratio 10/1 Assays

Vials	Diluent (µL)	Norvaline Sources		Diluted Norvaline Standards	
		Sources	Volume (µL)	Concentration (nmol/mL)	Volume (µL)
1	550	50µmol/mL	75	6000	361
2	264	Vial 1	264	3000	355
3	346	Vial 2	173	1000	360
4	371	Vial 3	159	300	355
5	175	Vial 4	175	150	350
Blank	350	Vial 5	0	0	350

Table 3: Preparation of Diluted Norvaline Standards for WR/Sample Ratio 1/1 Assays

Vials	Diluent (µL)	Norvaline Sources		Diluted Norvaline Standards	
		Sources	Volume (µL)	Concentration (nmol/mL)	Volume (µL)
1	2940	50µmol/mL	60	1000	1600
2	1400	Vial 1	1400	500	1600
3	1200	Vial 2	1200	250	1600
4	1200	Vial 3	800	100	1600
5	1200	Vial 4	400	25	1600
Blank	1600	Vial 5	0	0	1600

Note: The UV absorption responses may vary between the amines of proteins and Norvaline. To obtain more accurate assay results, a protein sample with known amount of reactive amines, if available, may be analyzed against Norvaline standards for the first time. Once their correlation is established, Norvaline may be routinely used as a convenient reference standard.

3 Preparation of Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:

   Total Volume of WR Required = (Blank + # of Standards + # of Unknowns) x (Volume of WR per Replicate) x (# of Replicates) x 1.05 (5% extra)

2. Prepare WR by mixing five parts of Reagent A and one part of Reagent B. Use the WR as soon as possible; sitting too long may cause loss of activity.

   For example, to conduct an assay of one blank, five standards and three unknown samples, if 250µL WR is required each in three replicates,

Total Volume of WR Required = (1 + 5 + 3) x 250µL x 3 x 1.05 = 7088µL

A sufficient amount of WR may be prepared by mixing 6mL Reagent A and 1.2mL Reagent B.

4 Assay Procedures

WR/Sample ratio of 10/1 or 1/1 may be used. The 10/1 ratio gives a wider analytical range, while 1/1 is a more sensitive method. For calculation of amine concentration in proteins, see Amine Estimation.

Note: For the general estimation of sample concentrations, modern plate readers or spectrophotometers normally have built-in software that automatically plots a linear or nonlinear regression line through the standard points, interpolates the samples, and reports the calculated values. Microsoft Excel may also be used to generate the standard curves, equations and estimate protein samples.

4.1 Microplate Assay Procedure

4.1.1 WR/Sample 10/1 in Microplate

The procedure has a linear range of 150-6,000nmol/mL for amine concentration, or 150-10,000µg/mL for protein concentration

1. Pipette 25µL of each Blank, standard and sample into a well.
   Note: Different sample sizes may be used as long as the WR/Sample is 10/1. If the sample size is small, e.g. 10µL, it is recommended to use half-area microplates to maintain the detection sensitivity.
2. Add 250µL WR into each well and shake gently. Cover the plate with parafilm.
3. Incubate for at least 15min at temperature between 25-37ºC.
4. Remove parafilm and measure UV absorbance in a plate reader at 350nm.
5. Subtract the average absorbance of the Blank from that of all other individual standard and unknown sample replicates. The Blank has to be measured every time alongside with the sample(s).
6. Generate a standard curve by plotting the Blank-corrected absorbance of each standard vs. its concentration, and estimate the amine concentration of each unknown sample, and then convert into the reactive amine density of the protein. See the section Amine Estimation.

4.1.2 WR/Sample 1/1 in Microplate

The procedure has a linear range of 25-1,000nmol/mL for amine concentration, or 25-2,000µg/mL for protein concentration.

1. Pipette 75µL of each Blank, standard and sample into a well.
2. Add 75µL WR into each well and shake gently. Cover the plate with parafilm.
   Note: Different sample sizes may be used, e.g. 125µL, as long as the WR/Sample is 1/1.
3. Incubate for at least 15min at temperature between 25-37ºC.
4. Remove parafilm and measure UV absorbance in a plate reader at 350nm.
5. Subtract the average absorbance of the Blank from that of all other individual standard and unknown sample replicates. The Blank has to be measured every time alongside with the sample(s).
6. Generate a standard curve by plotting the Blank-corrected absorbance of each standard vs. its concentration, and estimate the amine concentration of each unknown sample, and then convert into the reactive amine density of the protein. See the section Amine Estimation.

4.2 Test Tube Assay Procedure

4.2.1 WR/Sample 10/1 in Test Tube

The procedure has a linear range of 150-6,000nmol/mL for amine concentration, or 150-75,000µg/mL for protein concentration

1. Pipette 0.1mL of each Blank, standard and sample into the test tube or cuvette.
   Note: Different sample sizes may be used, e.g. 0.25mL, as long as the WR/Sample ratio is 10/1.
2. Add 1mL WR into each test tube and gently mix.
3. Incubate for at least 15min at temperature between 25-37°C.
4. Transfer to cuvette and measure UV absorbance at 350nm.
   Note: The test sample should be placed in the spectrophotometer chamber right before reading; continuous exposure to UV may affect the absorbance.
5. Subtract the average absorbance of the Blank from that of all other individual standard and unknown sample replicates. The Blank has to be measured every time alongside with the sample(s).
6. Generate a standard curve by plotting the Blank-corrected absorbance of each standard vs. its concentration, and estimate the amine concentration of each unknown sample, and then convert into the reactive amine density of the protein. See the section Amine Estimation.

4.2.2 WR/Sample 1/1 in Test Tube

The procedure has a linear range of 25-1,000nmol/mL for amine concentration, or 25-1,000µg/mL for protein concentration.

1. Pipette 0.5mL of each Blank, standard and sample into the test tube or cuvette.
   Note: Different sample sizes may be used, e.g. 1mL, as long as the WR/Sample ratio is 1/1.
2. Add 0.5mL WR into each test tube and gently mix.
3. Incubate for at least 15min at temperature between 25-37°C.
4. Transfer to cuvette and measure UV absorbance at 350nm.
   Note: The test sample should be placed in the spectrophotometer chamber right before reading; continuous exposure to UV may affect the absorbance.
5. Subtract the average absorbance of the Blank from that of all other individual standard and unknown sample replicates. The Blank has to be measured every time alongside with the sample(s).
6. Generate a standard curve by plotting the Blank-corrected absorbance of each standard vs. its concentration, and estimate the amine concentration of each unknown sample, and then convert into the reactive amine density of the protein. See the section Amine Estimation.

5 Compatibility and Interferent Removal

The X-Lyz™ Protein Amine Assay is compatible with reducing agents, common detergents, alcohols, urea, guanidine, metal chelators, and inorganic salts. When high concentrations of these substances are present, however, it is recommended to include them in the standards and Blank.

Primary amine-containing substances, such as amino acids, ammonia, and TRIS buffer interfere with the assay and is recommended to be removed. Non-primary amine buffers may be used to replace TRIS, such as HEPES, BICINE, BIS-TRIS, BIS-TRIS propane, and BIS-TRIS methane.

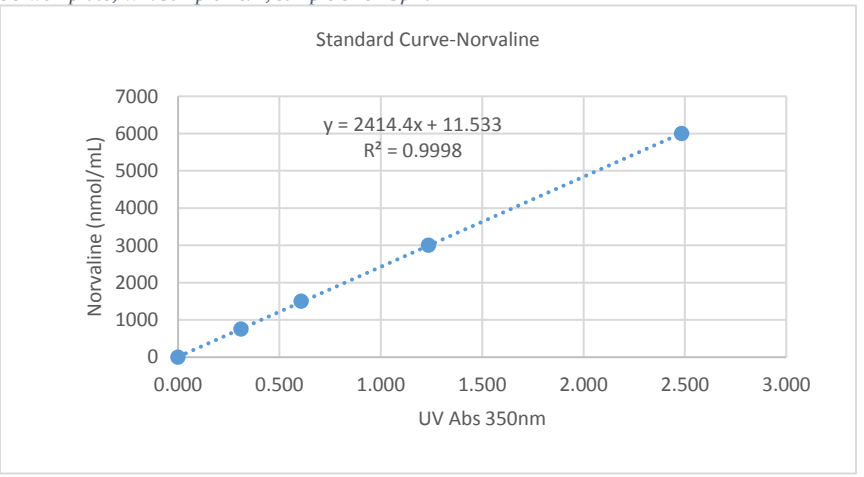
If necessary, follow the precipitation procedure below to remove the interferents and conduct assay.

1. Thoroughly mix an equal volume of 10% trichloroacetic acid aqueous solution with a protein sample for one minute in a centrifuge tube or a microplate (a microplate centrifuge is required).
2. Centrifuge the mixture (6,000 rpm/2,000 x *g* or higher) for 10min, decant and discard the supernatant.
3. Repeat Steps 1 and 2 to obtain a protein precipitate.
4. Dissolve the precipitate in a proper amount of WR, the volume in which the protein concentration should be no more than 700μg/mL.
5. Incubate for at least 15min at temperature between 25-37°C.
6. Read UV absorbance at 350nm.
7. The standard curve is generated using the procedure with WR/Sample 10/1 as described in the section Assay Procedures. Estimate a Nominal Concentration against the standard curve.
8. Use the formula below to calculate the reactive amine concentration of the original protein sample.
   Sample Amine Concentration = Nominal Concentration (Step 7) ÷ 11 x WR Volume (Step 4) ÷ Sample Volume (Step 1)

6 Amine Estimation

To estimate sample reactive amine concentrations, a standard curve is first plotted after measurement; the amine concentrations of unknown samples are then calculated from it. In Figure 1, the standard curve of Norvaline is generated and the linear equation Y = 2414.4X + 11.533 is obtained, where Y = amine concentrations and X = absorbance at 350nm. If an unknown protein sample, e.g., gives an absorbance of 1.000 (X), the sample's total reactive amine concentration (Y) is estimated be 2426nmol/mL (Y = 2414.4 x 1.000 + 11.533 = 2426nmol/mL). The concentration may be further converted into the reactive amine density by dividing it over the protein concentration in nmol/mL.

Figure 1: Standard Curve of Norvaline Concentration vs. Absorbance. The assay was conducted in 96-well plate, WR/Sample 10/1, sample size 25μL.



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